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ACUTE RESPONSES DURING BLOOD SUBSTITUTION IN THE CONSCIOUS RAT

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SUMMARY

1. Changes in the cellular components of blood in response to exchange transfusion with the fluorocarbon emulsion blood substitute Fluosol-DA 20% have been examined in conscious chronically catheterized rats.

2. Continuous isovolemic exchange transfusion at 1 ml . min^{-1} for 40 min reduced the mean haematocrit from 36% to less than 2%.

3. The animals behaved normally during blood replacement and no significant changes in mean arterial blood pressure, heart rate or respiration rate were observed.

4. An exponential decline in circulating red and total white cell count was observed during exchange transfusion. However, the rates of decline were significantly different for red and white cells as was reflected in the half-times ($t_{\frac{1}{2}}$) for the decrease in cell numbers (red blood cells: 7·5 min; white blood cells: 27·5 min) and the fractional turnover rates (k).

5. An exponential decline in total plasma protein and plasma albumin concentrations occurred in response to blood substitution with highly significant inverse log-linear relationships with time and with closely similar half-times ($t_{\frac{1}{2}}$) for the decrease in concentration (total protein: 10·5 min; albumin: 11·3 min) and the fractional turnover rates (k).

6. If a single well-mixed compartment is assumed for each type of blood cell and plasma proteins, the differences in turnover rates can be attributed to the volumes of these compartments (red blood cells: 10·9 ml.; white blood cells: 40·0 ml.; total protein: 15·2 ml.; albumin: 16·4 ml.). Possible anatomical boundaries for these compartments are considered.

7. Plasma sodium concentration showed a significant decrease in response to blood substitution; no corresponding changes in either plasma potassium or total plasma calcium concentrations were observed.

8. A progressive rise in plasma glucose concentration occurred during blood substitution, with levels at the end of the experiment approximately 2-fold higher than the mean initial value. There was also a small but not statistically significant decrease in plasma urea concentration during the procedure.

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INTRODUCTION

It is possible to replace the blood of experimental animals with totally synthetic perfluorocarbon-based whole blood substitutes (Geyer, 1973, 1975, 1978; Clark, 1978). The most important attribute of perfluorocarbon liquids as blood substitutes is their ability to combine reversably with large volumes of oxygen and carbon dioxide. Pure perfluorocarbon liquids are immiscible with water and are poor solvents for most physiological solutes, but one can use fine ($< 0{\cdot}1$ μm diameter) emulsions of perfluorocarbons in physiological electrolyte solutions. Preliminary observations of various physiological responses to blood substitution with these emulsions have been documented in several species including the rat (Geyer, 1975, 1978), rabbit (Kohno, Baba, Miyamoto & Niiya, 1979), dog (Takiguchi, Tsukada & Oyama, 1979), rhesus monkey (Ohyanagi, Sekita, Toshima, Kawa & Mitsuno, 1978) and human (Naito & Yokoyama, 1978). The results suggest that perfluorocarbon blood substitutes may be of great value in human and veterinary medicine and also in many areas of basic physiological research.

The previously published systematic investigations of the physiological responses to near total blood substitution appear to have been performed on anaesthetized animals, but since anaesthesia itself affects many physiological functions, it seemed important to re-examine the problem in conscious animals.

The experiments described here have investigated the acute changes in circulating red and white cell populations during continuous, isovolemic, exchange transfusion in conscious, unrestrained, chronically catheterized rats using the proprietary perfluorocarbon-based blood substitute: Fluosol-DA 20 % (Green Cross Corporation, Osaka, Japan). Since the liver and other tissues contain considerable amounts of proteins which may under certain circumstances be released into the circulation (see e.g. Rothschild, Oratz & Schreiber, 1976) it might be expected that there would be differences in the rate at which various plasma proteins are removed from the circulation in response to blood substitution. Therefore the acute changes in total plasma protein concentration and plasma albumin concentrations have also been measured together with the changes in various plasma electrolyte and metabolite concentrations.

Some of these results have been published previously in a preliminary form (Lowe, McNaughton & Hardy, 1982).

METHODS

Animals

Mature female Sprague–Dawley/OLA rats (mean $\pm$ S.E. of mean body weight: 279 ± 4 g) were used in these experiments. The animals were kept in cages in the laboratory animal house and maintained on a diet of Dixon's FFG (M) food concentrate pellets (Dixon's Ltd., Ware) *ad libitum*. The temperature and lighting regimes were regulated so as to maintain prevailing conditions of 20–25 °C and 14 hr light/day (lights on: 07.00–21.00 hr). Food was withheld for at least 24 hr before surgery; however, free access to water was allowed at all times.

Experimental procedures

The animals were initially anaesthetized with ether, weighed and then given a single 2·0 ml . kg^{-1} intraperitoneal injection of the barbiturate-based anaesthetic: Equithesin (Green, 1969).

The animal was placed in a supine position and the femoral artery and vein were exposed in the inguinal region. Specially constructed polypropylene catheters consisting of 1·5 cm length of Portex PP25 (i.d. 0·40 mm; o.d. 0·80 mm; Portex Ltd. Hythe) heat welded onto a 50 cm length of Portex PP100 (i.d. 0·86 mm; o.d. 1·52 mm) were inserted into the right femoral artery and vein. The distal ends of the catheters were passed beneath the skin from the site of the incision to the back of the neck by means of a hollow metal trochar. At this point they entered a harness and swivel arrangement modified from that described by Weeks & Collins (1964). The catheters were sealed using three-way stop-taps and filled with physiological saline (0·9 % NaCl w/v) containing 1000 i.u. heparin . ml.$^{-1}$. Following catheterization, the incision was sprayed with Rikospray (Bacitracin: 375,000 i.u.; Polymixin: 150,000 i.u., Neomycin: 500,000 i.u.; Riker Laboratories, Loughborough) and the skin was closed with cotton sutures. The animals were given a 0·1 ml. intramuscular injection of 25 % v/v Penidural (300,000 i.u., Benzathine penicillin . ml.$^{-1}$, Wyeth Laboratories, Maidenhead) in distilled water. At least 24 hr were allowed for recovery from surgery and anaesthesia.

TABLE 1. Composition of Fluosol-DA 20 (w/v %)

Perfluorodecalin	14·0*
Perfluorotripropylamine	6·0*
Pluronic F-68	2·7†
Yolk phospholipids	0·4†
Glycerol	0·8
NaCl	0·600
KCl	0·034
$MgCl_2$	0·020
$CaCl_2$	0·028
$NaHCO_3$	0·210
Glucose	0·180
Hydroxyethylstarch	3·0‡

Total osmotic pressure: 410 m-osM; total oncotic pressure: 380–395 mm H_2O. * Gas-carrying emulsion. † Emulsifying agents. ‡ Oncotic pressure.

Isovolemic exchange transfusion was performed with the animals contained in specially constructed, transparent, gas-tight chambers. The blood substitute: Fluosol-DA 20 % (see Table 1) was warmed to 38 °C and infused into the venous catheter at a rate of 1 ml . min^{-1} using a continuous infusion LKB Varioperpex pump (LKB Instruments, Selsdon) while blood was simultaneously removed at the same rate from the arterial catheter using a second similar pump. The arterial effluent was collected serially using an LKB Ultrarac II fraction collector. Blood pressure and heart rate were monitored at 10 min intervals by means of a Bell & Howell type 4-422-001 pressure transducer connected to the arterial catheter; readings were displayed on an Ormed M19 Recorder (Ormed Engineering, Welwyn Garden City). Respiration rate was monitored by direct observation. During exchange transfusion, the oxygen concentration in the chamber was increased to 80 % and stabilized there using an oxygen-sensitive probe and an Electrodyne IMI 3700 oxygen controller (Becton Dickinson, Wembley) connected to a flowmeter assembly. Water and carbon dioxide were absorbed by silica gel and soda lime respectively, which were contained in separate porous bags suspended within the cage. The animals were allowed free access to food and water throughout the procedure. At the end of the perfusion, the animals were given a supplementary infusion of 1·5 ml. Fluosol-DA . 100 g^{-1} body weight to aid survival (Watanabe, Hanada, Yano, Yokoyama, Suyama & Naito, 1979).

Analytical procedures

Samples of blood or arterial effluent (1 ml.) were collected into plastic tubes containing anticoagulant (Sarstedt, Leicester). The haematocrit and 'fluorocrit' (Ohyangi *et al.* 1978) were measured using an automatic Adams 'Autocrit' microhaematocrit centrifuge (Clay Adams, Parsippany, U.S.A.). Erythrocyte and total white cell counts were measured using an automatic ZBI Coulter Counter (Coulter Electronics, Harpenden); samples were diluted using 'Isoton II'. Manual counts for blood cells were performed on selected samples to confirm measurements

obtained using the Coulter Counter. Differential white blood cell counts were obtained following staining of blood smears with Leishman's stain; a minimum of 500 cells were counted manually.

For chemical analysis samples were centrifuged at 2500 r.p.m. for 30 min at +4 °C and the plasma or supernatant removed and then stored at −20 °C. Total plasma protein concentration was measured using the Folin Phenol reagent method described by Lowry, Rosebrough, Farr & Randall (1951); the within and between assay coefficients of variation were 3·3 and 6·8% respectively. Plasma albumin concentration was estimated by the bromocresol green binding method of Doumas & Biggs (1972) as described by Kachmar & Grant (1976). Plasma sodium and potassium concentrations were estimated using a Corning 455 flame photometer; total plasma calcium concentration was estimated by means of the orthocresolphthalein complexone method without sample deproteinization (Sarker & Chauhan, 1967). Plasma glucose concentration was estimated using the hexokinase method (Schmidt, 1961), and plasma urea concentration using the urease assay method described by Gutmann & Bergermeyer (1974).

Statistical analyses were made according to the methods of Snedecor & Cochran (1967); statistical significance between mean values was assessed using the conventional Student's t test or paired t test accordingly.

RESULTS

Behavioural, cardiovascular and respiratory responses during exchange transfusion

In the series of experiments reported here forty-three rats were subjected to progressive isovolemic exchange transfusion at a rate of 1 ml . min^{-1} for 40 min. All the animals tolerated this procedure well and behaved in an apparently normal manner throughout, moving freely about their cage and taking food and water. None of the animals showed any obvious signs of distress during blood replacement; this normal behaviour was also reflected in the fact that no significant ($P > 0·1$) changes were observed in heart rate, mean arterial blood pressure and respiration rate during blood substitution (Fig. 1).

Blood cell changes during exchange transfusion

As would be expected, the progressive dilution of blood by the infusion of Fluosol-DA resulted in a gradual fall in the blood cell population, as monitored by the haematocrit (Fig. 2). The final haematocrit following 40 min of continuous exchange transfusion was 1·7 ± 0·1%. These changes were accompanied by an increase in the amount of Fluosol-DA in the circulation, as assessed by the fluorocrit, up to a steady-state plateau level of 17·6 ± 0·7% at the end of the perfusion (Fig. 2); this fluorocrit corresponded to the proportion of emulsion particles present in pure Fluosol-DA 20% when spun in the same haematocrit centrifuge (*ca.* 17–18%).

An exponential decline in both erythrocyte and total white blood cell numbers occurred during blood substitution (Fig. 3); however, the circulating red and white cell populations decreased at different rates in response to this procedure: the changes in red and white cell numbers during exchange transfusion showed a highly significant ($P < 0·001$) inverse log-linear relationship with time with $r > 0·9$ in both cases. However, the estimated half-time ($t_{\frac{1}{2}}$) of the decrease in cell numbers, as derived from a log-linear plot (Fig. 3) was different from the red and white cell populations (red cells: 7·5 min, white cells 27·5 min). These differences were reflected in the fractional turnover rates (k) derived from the $t_{\frac{1}{2}}$ values ($k = \log_e 2/t_{\frac{1}{2}}$: red cells 0·09; white cells 0·03).

The fact that white cell numbers declined at a much slower rate than did the red cells may be in part attributed to the mobilization of lymphocytes, which constituted

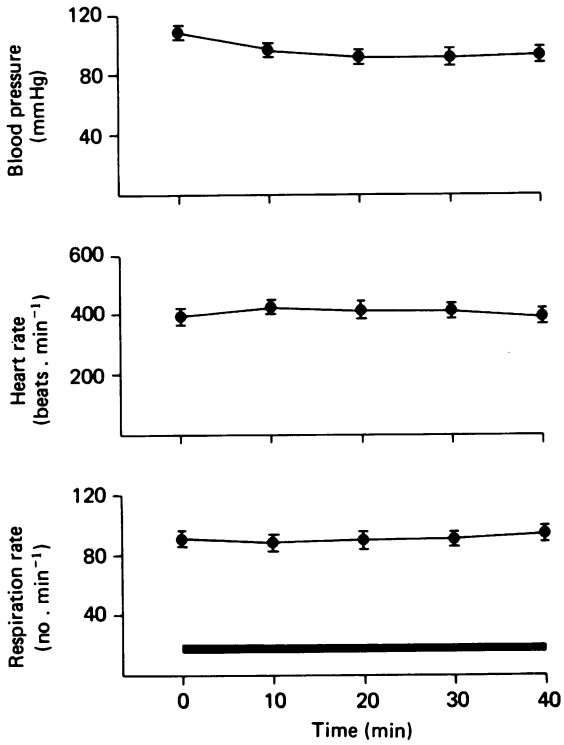

Fig. 1. Changes in mean arterial blood pressure, heart rate and respiration rate in rats exchange transfused with Fluosol-DA 20%. Each point represents the mean of 32–43 observations. Vertical bars represent S.E. of mean. Horizontal bar: duration of exchange transfusion.

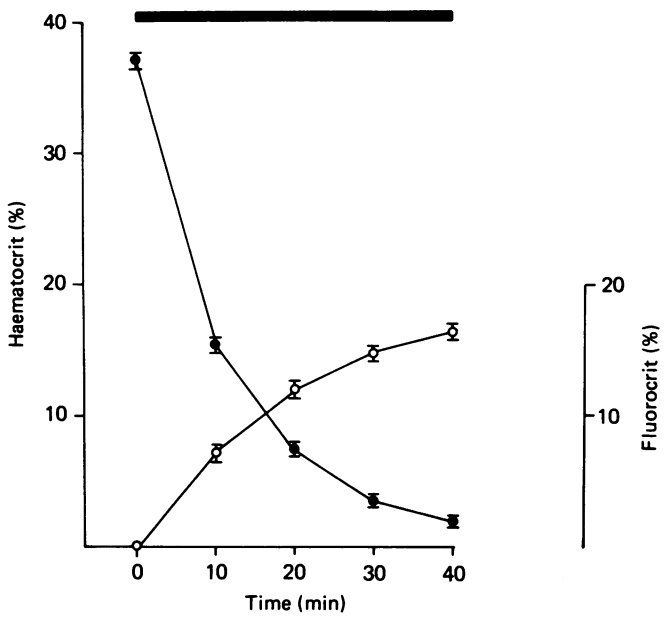

Fig. 2. Changes in haematocrit (●) and 'fluorocrit' (○) in rats exchange transfused with Fluosol-DA 20%. Vertical bars represent S.E. of mean (n = 43). Horizontal bar: duration of exchange transfusion.

a much larger proportion of the total remaining white cell population in animals after blood substitution as reflected by a differential white cell count (Fig. 4). In contrast, a fall in neutrophil numbers occurred during blood replacement with no corresponding changes in the remaining white cells.

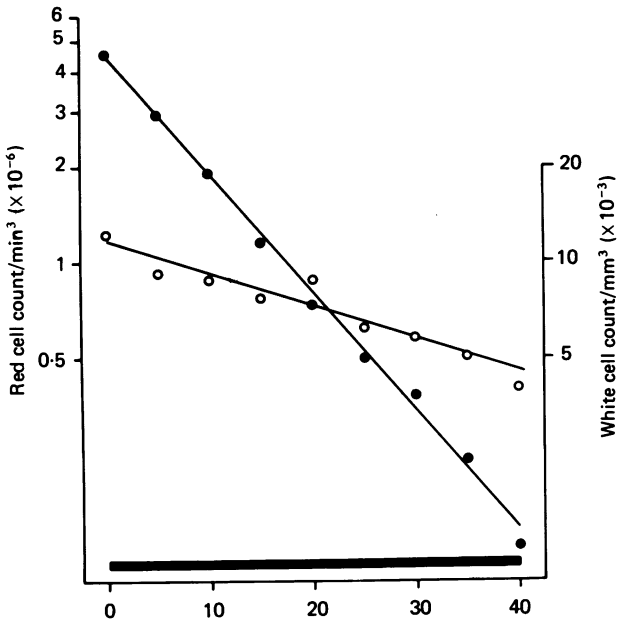

Fig. 3. Semi-logarithmic plots of the changes in the circulating red blood cell count (●) and white blood cell count (○) in rats exchange transfused with Fluosol-DA 20%. Each point represents the mean of eight observations. (Lines show the best fit by the method of least squares.) Horizontal bar: duration of exchange transfusion.

Plasma protein changes during exchange transfusion

The dilution of blood during exchange transfusion resulted in a pronounced and progressive decrease in both the total plasma protein and plasma albumin concentrations as reflected by a log-linear plot (Fig. 5). The estimated half-time ($t_{\frac{1}{2}}$) of the decrease in total plasma protein concentration and albumin concentration showed good agreement at 10·5 and 11·3 min respectively as did the corresponding fractional turnover rates (0·07, 0·06).

Plasma electrolyte and metabolite changes during exchange transfusion

Exchange transfusion of rats with Fluosol-DA 20% was not accompanied by any significant changes in plasma potassium or total calcium concentrations measured before and immediately following blood substitution (Table 2). However, plasma sodium concentration showed a significant decline ($P < 0{\cdot}02$) in response to the blood replacement procedure (Table 2).

A small, but not statistically significant fall ($P > 0{\cdot}1$) in plasma urea concentration was observed in response to blood substitution. In contrast, plasma glucose concentration showed a progressive rise during exchange transfusion; the mean glucose concentration at the end of the blood replacement procedure was almost double the initial pre-perfusion concentration (Table 2).

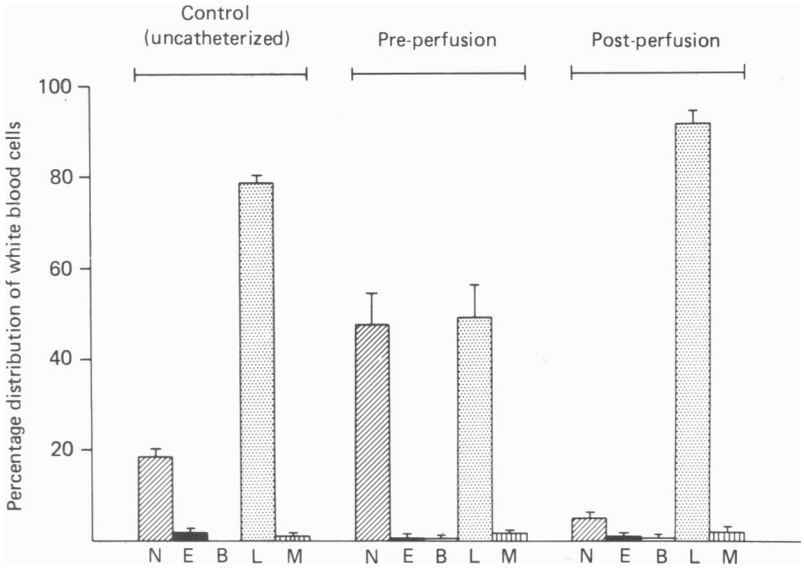

Fig. 4. The percentage distribution of circulating white blood cells in control (uncatheterized) rats and chronically catheterized animals before and immediately after exchange transfusion with Fluosol-DA 20%. Vertical bars represent S.E. of mean ($n = 6$). N = neutrophil, E = eosinophil, B = basophil, L = lymphocyte, M = monocyte.

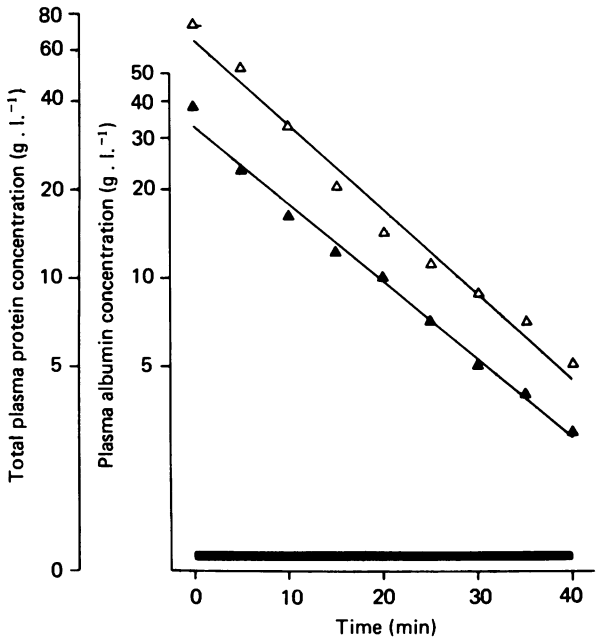

Fig. 5. Semi-logarithmic plots of the changes in total plasma protein concentration (▲) and plasma albumin concentration (△) in rats exchange transfused with Fluosol-DA 20%. Each point represents the mean of seven observations. (Lines show the best fit by the method of least squares.) Horizontal bar: duration of exchange transfusion.

TABLE 2. Effect of blood replacement with Fluosol-DA 20% on plasma electrolyte and metabolite concentrations (m-mole $l.^{-1}$) in the rat

	Pre-perfusion	Post-perfusion
Sodium	143·9 ± 0·8 (8)	137·9 ± 1·9 (8)*
Potassium	4·6 ± 0·2 (8)	4·2 ± 0·2 (8)
Calcium	2·3 ± 0·1 (8)	1·8 ± 0·2 (7)
Urea	4·0 ± 0·6 (5)	2·9 ± 0·3 (5)
Glucose	8·1 ± 0·3 (7)	15·5 ± 1·8 (7)**

Values are mean ± S.E. of mean with number of animals in parentheses. *$P < 0.02$; **$P < 0.001$.

DISCUSSION

The remarkable ability of fluorocarbon emulsions to act as substitutes for whole blood has been the subject of much experimental analysis during the last decade (see, e.g. Geyer, 1975, 1978, 1979). In particular, great interest has focused upon the survival and recovery of so-called 'bloodless' animals; i.e. animals, usually rats, in which progressive blood replacement has been continued until the haematocrit is less than 1%. However, the fact that concern with long-term survival has overshadowed other aspects of experimental studies on blood substitution has meant that the changes occurring *during* blood substitution have been virtually completely neglected: this paper represents an attempt to analyse some of these changes.

In order to follow changes during blood substitution, a technique has been developed which enables the procedure to take place in conscious animals and which also permits precise assessment of the progressive changes in the intravascular fluid, by fractionating the arterial effluent.

In control studies, rats with intravascular catheters and harnesses have been kept for many days in experimental chambers closely similar to those used in the present experiments: such animals show no evidence of discomfort, apart from the slight encumbrance of the harness. It therefore seems probable that the experimental rats used in these studies provided an acceptable physiological base line from which to assess the changes associated with blood substitution.

It is noteworthy that the behaviour of the rats used in this work remained normal during blood substitution: they moved freely about the cage and took food and water in an apparently unconcerned manner. There were also no significant changes in arterial blood pressure, heart rate or respiration rate during this period (Fig. 1). These results represent the first such observations on conscious animals during severe blood substitution and are thus of particular interest, since they provide information about central nervous function during the transition from red blood cell gas carriage to fluorocarbon gas transport. The fact that behavioural disturbance was minimal can be taken to indicate that the functioning of higher centres was unimpaired, while the stability of cardiovascular and respiratory indices implies normal brainstem activity. Therefore, if it is assumed that the C.N.S. is the tissue most vulnerable to adverse changes in its local *milieu*, it can be concluded that, in the short term at least, virtually total replacement of blood by Fluosol-DA can take place with minimal homeostatic disturbance. Thus, despite the dramatic changes in the composition of the intravascular fluid during the 40 min period of blood substitution, essentially normal brain function was maintained.

Fluosol-DA contains no blood cells; it would therefore be expected from first principles that blood substitution with Fluosol-DA at a constant rate, as in these experiments, should result in a progressive flushing-out of blood cells, the numbers of which should thus fall exponentially. It can be seen from Fig. 3 that this is indeed the case, for both red and white cell numbers decrease with a highly significant inverse linear relationship when plotted semi-logarithmically with respect to time. However, it is also clear from Fig. 3 that the slopes of the two lines are different: this is significant and results in a marked discrepancy in the calculated values for the half-time for decrease in the respective cell numbers ($t_{\frac{1}{2}}$) and fractional turnover rate (k) (see Results).

The simplest hypothesis to explain these results is to assume a single, well-stirred compartment for each component, in which case the differences would reflect the size of each compartment and the initial volume of the red cell compartment ($V_0 = k/1$ ml. min^{-1}) would be 10·9 ml., while that of the white cells would be 40·0 ml. The value for the red cell compartment is in fact slightly larger than the expected red cell space of approximately 8 ml., calculated from published figures for plasma volume (Trippodo, Walsh & Frohlich, 1978), assuming a haematocrit of 37 % and a body weight of 270 g. However, this discrepancy could readily be explained by the extravascular red cell pools in the spleen and bone marrow. The calculated value of 40 ml. for the white cell compartment is less easy to ascribe: it is in fact close to the predicted volume of the extracellular compartment in a 270 g rat (43 ml.), although clearly in practice there would not be a uniform white cell population throughout this volume. The discrepancy must therefore presumably be attributed to the effect of reservoirs of high lymphocyte concentration such as lymph nodes: this suggestion is supported by the increase in the relative lymphocyte population noted post-perfusion (Fig. 4).

Measurements of total plasma proteins and albumin during blood substitution yielded estimates of the volumes of the respective compartments of 15·2 and 16·4 ml. These values are reasonably in accord with an expected plasma volume of 13·5 ml. for a 270 g rat, assuming a plasma volume of 5 ml. 100 g^{-1} (Trippodo *et al.* 1978). This implies there was relatively little exchange with interstitial fluid proteins or other extravascular protein sources within the time course of the experiment.

As far as blood cells are concerned, the single-compartment hypothesis outlined above is clearly simplistic since it ignores the contribution made to the system by continuous production from haemapoietic tissues. Nevertheless, it seems a reasonable working hypothesis which should be susceptible to future experimental analysis and which moreover receives some further support from the studies of plasma protein kinetics.

With regard to the changes observed in the plasma concentrations of substances also present in the Fluosol-DA itself: there were no significant changes in potassium, or total calcium. The former will not be discussed further, but in the case of calcium, the progressive elimination of potential binding proteins during perfusion would be expected to cause profound changes in the proportion of the total calcium present in the ionized state: this is currently being investigated further. There was a significant fall in the sodium concentration and a significant increase in glucose concentration (Table 2). The fall in sodium concentration can most simply be

explained by the fact that the sodium concentration in Fluosol-DA is 130 m-mole (Table 1) which is somewhat less than the mean value of approximately 144 m-mole . $l.^{-1}$ in the plasma of rats prior to perfusion (Table 2). Thus infusion of 40 ml. of effectively hyponatric fluid would be expected to produce a fall in plasma sodium concentration of the order of that observed.

In the case of glucose, the increase in plasma glucose concentration from about 8 m-mole . $l.^{-1}$ to approximately double that value (Table 2) could not be accounted for by the additional glucose present in Fluosol-DA. The concentration there (10 m-mole . $l.^{-1}$; Table 1) would clearly be insufficient to explain the pronounced post-perfusion hyperglycaemia, which must, therefore, in part, be due to the mobilization of endogenous glycogen stores, as would be expected consequent upon the stress of the perfusion procedure.

The observation that urea concentration did not change during perfusion is difficult to explain, although an early report (Schmidt-Nielsen, 1955) claiming that blood loss acts to decrease urea clearance could provide a possible mechanism. However, since exchange transfusion in the present experiments was performed under strict isolvolemic conditions, it is unlikely that the stimulus to urea release and/or its decreased clearance is provided by changes in intravascular fluid volume itself.

In view of the profound changes in the composition of intravascular fluid described in this paper, it is perhaps remarkable that the animals tolerate severe blood substitution with Fluosol-DA in the short term.

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